Bovine Lumpy Skin Disease Virus Antibody ELISA Kit

Catalog No: E-AD-E132

96T/96T*2/96T*5

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit is comprised by HRP conjugate, other reagents and ELISA Microtiter plate pre-coated with recombinant Bovine Lumpy Skin Disease Virus (LSDV) antigen. Apply the principle of enzyme-linked immunoassay (ELISA) to detect LSDV-Ab in serum of porcine. During the experiment, add control and samples into the ELISA Microtiter plate, LSDV-Ab will be bound with the antigen on the ELISA Microtiter plate. Then horseradish peroxidase (HRP) conjugate is added to each ELISA Microtiter plate well. The unbound HRP Conjugate will be removed by washing and substrate reagent is added for color development. At last, end the reaction by adding Stop Solution to produce a yellow product. There is a positive correlation between the OD value of samples and the concentration of LSDV-Ab. Measure the absorbance value of each well by using a microplate reader with 450 nm (630 nm) wavelength, then we can judge whether LSDV antibody exist in the sample.

Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	10 mL
Sample Diluent	6 mL
20×Concentrated Wash Buffer	25 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	0.5 mL
Negative Control	0.5 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ incubator or water bath
Deionized or distilled water
Absorbent paper

Notes

1. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly performed. All the waste should be handled as contaminant.
2. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contacted carelessly.
3. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
4. Concentrated wash buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature in order to dissolve completely before use.
5. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
6. The tested sample should keep fresh.
7. The results shall depend on the readings of the microplate reader.
8. **Each reagent is optimized for use in the E-AD-E132. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-AD-E132 with different lot numbers**.
9. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Storage and expiry date

Store at 2-8℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum/Plasma:** Use the conventional method to prepare serum and plasma, the serum and plasma must be clear, no hemolysis and no pollution. Samples can be conserved at 2-8℃ in 1 week, and it should be stored at - 20℃ for a long term storage.
   Note: Collection tubes should free of pyrogens and endotoxin.
2. **Cell supernatant**: Centrifuged at 3000 rmp for 10 min to remove particles and polymers, frozen at -20 ℃ and avoid repeated freezing and thawing.
3. **Tissue homogenate**: Add the tissue to an appropriate amount of physiological saline and homogenize it. Centrifuge at 3000 rpm for 10 min and collect the supernatant, frozen at -20 ℃ and avoid repeated freezing and thawing.
4. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 2 wells for negative/positive control respectively. **Samples need test in duplicate.**
2. **Add control:** add 50 μL of **positive/negative control** to positive/negative control well.
3. **Add sample:** add 10 μL of sample, and then 40μL of **Sample Diluent**.
4. **HRP conjugate:** add 100 μL of **HRP Conjugate** into each sample and control well, cover the plate sealer and incubate at 37℃ for 60 min.
5. **Wash:** remove the liquid in each well. Immediately add 350 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
6. **Color Development:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** into each well. Cover the plate sealer and mix thoroughly, incubate at 37℃ for 15 min in shading light.
7. **Stop reaction:** add 50 μL of **Stop Solution** into each well, mix thoroughly.
8. **OD Measurement:** measure the absorbance value (A-value) of each well by using a Microplate Reader with 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) wavelength.

Reference value

Normally, average absorbance of negative control ≤ 0.15 and average absorbance of positive control ≥ 1.0.

Interpretation of the results

1. Cut off = average A value of negative control + 0.15
2. Positive result: A_{450} ＞ Cut off
3. Negative result: A_{450} ＜ Cut off